

J Muscle Res Cell Motil (2008) 29:159–162
DOI 10.1007/s10974-009-9169-x

REVIEW PAPER

Myocardial adaptations in the failing heart: cause or consequence?

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Received: 4 December 2008 / Accepted: 7 January 2009 / Published online: 14 February 2009
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Abstract Many changes in morphology, biochemical properties and myocyte function occur during development to heart failure. Most changes may be compensatory, yet unable to prevent cardiac dysfunction in the long run. This illustrates that it is important to carefully dissect the disease causing modifications from cardiac adaptation, in order to obtain a better understanding of the pathophysiological processes leading to heart failure.

Keywords Heart failure · Myocyte contractility · Protein phosphorylation

Introduction

Heart failure is a disease with a broad clinical presentation and a complex underlying pathophysiology. A variety of cardiac diseases can damage the heart and trigger the process leading to heart failure (Francis 2001). Although the exact mechanisms involved in the progression towards heart failure are not yet fully understood, the involvement of many mechanical, biochemical and genetic factors has become evident over the recent years.

Structural remodelling of the heart, including hypertrophy, apoptosis and slippage of myocytes and altered extracellular matrix turnover, is frequently observed in heart failure. Remodelling often occurs in response to a primary defect, such as a myocardial infarction or

hypertension, and is frequently acknowledged to contribute to ventricular dysfunction (Francis 2001; Radauceanu et al. 2008; Yankey et al. 2008).

Several biochemical factors, e.g. cytokines and neurohormones, have been shown to be associated with heart failure (Mahmoudabady et al. 2008; Ratnasamy et al. 2008; Torre-Amione et al. 1996; van Empel and De Windt 2004). Overexpression of TNF- α contributes to cardiac remodelling by inducing hypertrophy and apoptosis of myocytes. In transgenic mice models this overexpression induces heart failure (Bryant et al. 1998). Activation of the renin-angiotensin system worsens the progression of heart failure by promoting water and salt retention and vasoconstriction, thereby raising blood pressure (Ram 2008). Furthermore, angiotensin II is implicated to exert a direct effect on remodelling by stimulating cardiomyocyte hypertrophy and extracellular matrix production (Billet et al. 2008). Sympathetic nervous activity is elevated in heart failure to maintain cardiac output, which is reflected by high levels of catecholamines. Overstimulation of the sympathetic system leads to desensitisation and downregulation of β -adrenergic receptors in heart failure (Bristow et al. 1993). The natriuretic system influences hemodynamics and variations in this system are associated with increased risk for cardiovascular disease (Lanfear 2008).

Genetic factors involved in heart failure are becoming increasingly clear both in hypertrophic (HCM) and dilated cardiomyopathy (DCM). HCM is characterized by thickening of the septum and left ventricular wall without an underlying cardiac or systemic defect. HCM is a heterogeneous disease with a wide range of clinical presentations. Familial HCM (FHCM) is the most frequent inheritable cardiac disease, caused by mutations in genes encoding for several sarcomeric proteins (Maron 2004). DCM is characterized by an increased left ventricular volume and a

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reduced ejection fraction (systolic failure). Although mutations, some of which located in the sarcomeric proteins, have been found to cause DCM, mostly the cause is unknown. It has been proposed that HCM eventually evolves to DCM, although there is also evidence suggesting dissimilar pathomechanisms for HCM and DCM without a common endpoint.

Myocyte contractility in heart failure

Cardiac function is largely determined by the contractile properties of the sarcomere. During contraction, the myosin of the thick filament and the actin of the thin filament interact. A rise in intracellular free Ca^{2+} initiates contraction by binding of Ca^{2+} to troponin C. As a result, the tropomyosin-troponin complex changes its conformation, thereby unblocking the myosin binding site on actin and allowing crossbridge formation. A number of sarcomeric proteins are involved in the regulation of contraction (reviewed by de Tombe 2003).

Several changes in expression of proteins involved in contraction have been found in heart failure, such as a decreased α -myosin heavy chain expression, a shift in titin isoform from the shorter N2B to the longer, more compliant N2BA isoform (McDonald and Herron 2002; Nakao et al. 1997).

Troponin I and myosin binding protein C phosphorylation in heart failure

The function of many cardiac sarcomeric proteins is regulated by post translational modifications, in particular phosphorylation (for a recent review, see, Jin et al. 2008). The foremost kinases active in the heart are protein kinase A (PKA), protein kinase C (PKC) and Ca^{2+} -dependent calmodulin kinase 2 (CamK2). The phosphorylation status of sarcomeric proteins is influenced by phosphatase activity of protein phosphatases 1 and 2A as well.

PKA is activated upon β -adrenergic stimulation and is a key player in cardiac adaptation to increased cardiac demand. The main sarcomeric protein targets of PKA dependent phosphorylation are cardiac troponin I (cTnI), myosin binding protein C (cMyBP-C) and titin. PKC phosphorylates several sites on cTnI, cardiac troponin T (cTnT) and cMyBP-C. Several studies have reported a downregulation of PKA and an upregulation of PKC in heart failure (LeWinter 2005; Sumandea et al. 2004).

Phosphorylation of cTnI by PKA is associated with reduced Ca^{2+} -sensitivity and increased crossbridge kinetics, enabling fast relaxation and so maintaining adequate

diastolic function at elevated cardiac output (Hamdani et al. 2008; LeWinter 2005). Ample evidence suggests a role for PKA dependent activation of cMyBP-C in the kinetics of force development. In mice, treatment of skinned ventricular myocardium with PKA accelerated stretch activation, an effect not seen in myocardium from cMyBP-C knock out mice (Stelzer et al. 2007). The functional consequences of cMyBP-C phosphorylation by PKC are less well studied (Lim et al. 1985; Xiao et al. 2007).

Altered cTnI and cMyBP-C phosphorylation in human heart failure

Research on the pathophysiology of inheritable cardiomyopathies is mainly conducted in animal models. Additionally, the functional consequences of FHCM causing mutations are largely studied in vitro, using (parts of) exogenous protein. Human studies in more physiological circumstances are scarce but the delicate regulation of the sarcomere, e.g. by phosphorylation, is currently subject of intense study (El Armouche et al. 2007; Jacques et al. 2008). Phosphorylation of PKA-target proteins cTnI and cMyBP-C appears to be reduced in myocardium of end-stage heart failure patients (Bodor et al. 1997; Messer et al. 2007; van der Velden et al. 2006). Less is known about the phosphorylation of these proteins in FHCM.

Hence, we investigated whether the phosphorylation status of sarcomeric proteins in FHCM differs from that in idiopathic DCM (IDCM) and non-failing donor samples. Sarcomeric proteins were separated on a gradient gel and stained with ProQ Diamond and SYPRO Ruby stain to determine phosphorylation and expression level, respectively, as described previously (Zaremba et al. 2007). In accordance with previous studies, we demonstrated a reduced phosphorylation of cTnI and cMyBP-C in IDCM ($n = 12$) compared to non-failing donor samples ($n = 10$) by 87 ± 3 and $55 \pm 5\%$, respectively (Fig. 1). The phosphorylation level of cMyBP-C was lowered in FHCM patients ($n = 25$) compared to donors as well (0.64 ± 0.07 vs. 1.0 ± 0.04 , respectively, $P < 0.05$ in one-way ANOVA followed by post-test Bonferroni analysis; Fig. 1), although not to the extent observed in IDCM samples. Considerable variation in cMyBP-C was observed in the FHCM samples, underlining the heterogeneous character of FHCM. Therefore, we analysed phosphorylation in a more homogenous subgroup of patients with a mutation in *MYBPC3*, the gene encoding cMyBP-C. Surprisingly, cMyBP-C phosphorylation was alike in donor and *MYBPC3* mutant FHCM samples (data not shown). cTnI phosphorylation was lowered considerably by $86 \pm 4\%$ in FHCM patients compared to donor, similar to the reduction observed in IDCM patients.

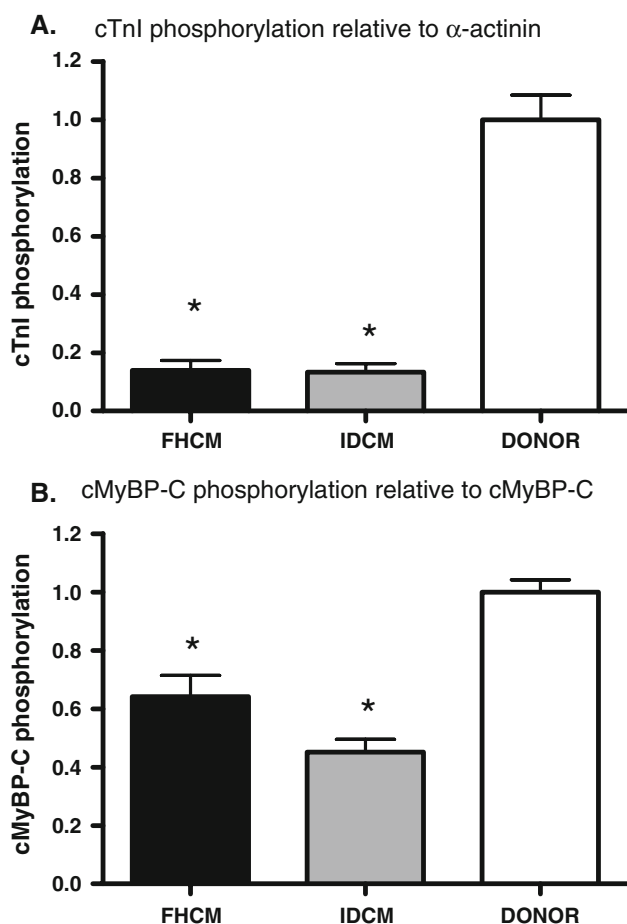


Fig. 1 Phosphorylation status of cTnI and cMyBP-C. **a** ProQ Diamond staining of SDS-PAGE gels showed that the phosphorylation status of cTnI (relative to the intensity of the SYPRO Ruby stained α -actinin band) was lower in IDCM ($n = 12$) and FHCM ($n = 25$) compared to donor ($n = 10$) samples. **b** The phosphorylation status of cMyBP-C (relative to the intensity of the SYPRO Ruby cMyBP-C band) was lower in IDCM than in donor, but comparable between FHCM and donor samples. All values of IDCM and FHCM are given relative to values found in donor, which were set to 1. * $P < 0.05$ failing versus donor in one-way ANOVA followed by post-test Bonferroni analysis

Conclusions

Careful investigation of changes in morphology, biochemical profile and genetics of heart failure patients is required to enhance our understanding of the pathophysiology of heart failure and of myocyte function in general. It will be difficult to make a distinction between pathological adaptations and compensatory mechanisms, because a number of changes occur simultaneously. We demonstrated divergent phosphorylation of cTnI and cMyBP-C in distinct patient groups. Furthermore, the high variability observed even within the FHCM patient group emphasises the wide range of adaptations that occur during heart failure.

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